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Review

SOCS regulation of the JAK/STAT signalling pathway

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ABSTRACT

The suppressor of cytokine signalling (SOCS) proteins were, as their name suggests, first described as inhibitors of cytokine signalling. While their actions clearly now extend to other intracellular pathways, they remain key negative regulators of cytokine and growth factor signalling. In this review we focus on the mechanics of SOCS action and the complexities of the mouse models that have underpinned our current understanding of SOCS biology.

Contents

1. Introduction

Regulation of the hematopoietic system and the immune response is largely mediated by small-secreted proteins called cytokines. Cytokine signalling is initiated through ligand interaction with specific trans-membrane receptor subunits. The subsequent receptor oligomerisation results in activation of either an intrinsic kinase domain or receptor associated JAK kinases, and the following cascade of intracellular phosphorylation and signal transduction culminates in an appropriate cellular response [1]. However, this cascade requires exquisite cellular control and loss of regulation can promote tumorigenesis and chronic

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inflammation. It is therefore not surprising that multiple tiers of control have evolved to contain cytokine signalling. The threshold, magnitude and specific responses elicited by cytokine stimulation are regulated by numerous mechanisms including tyrosine phosphatases, receptor internalisation, proteasomal degradation of signalling adaptor molecules, soluble receptor antagonists and specific inhibitors, including the PIAS and suppressor of cytokine signalling (SOCS) proteins. The expression of SOCS proteins can be induced by cytokine stimulation, and they serve to interfere with signalling not only from the inducing cytokine in a classic "negative-feedback" loop, but also to regulate signalling downstream of other cytokines, a process known as "cross-talk".

1.1. Discovery of the SOCS proteins

Three groups independently discovered the SOCS1 protein: as a JAK binding protein (JAB) [2]; as a suppressor of IL-6 signalling (SOCS1) [3]; and based on sequence homology with the Stat3-SH2 domain (STAT-induced STAT inhibitor; SSI) [4]. It was subsequently shown to belong to a family of SH2 domain-containing proteins (SOCS1-7, CIS). While SOCS1 may be the archetypal SOCS protein, it was historically preceded by CIS (cytokine inducible SH2-containing protein). CIS was discovered as an IL-3-inducible gene and overexpression studies suggested that it inhibited signalling by competing for binding to Stat5 recruitment sites within the IL-3β, EPO, prolactin, and GM-CSF receptor cytoplasmic domains [5,6].

1.2. Critical roles of SOCS proteins

Over the past decade, SOCS proteins have been implicated in the regulation of over 30 cytokines, including interleukin (IL)-6, leukemia inhibitory factor (LIF), leptin, granulocyte colony-stimulating factor (G-CSF), IL-10, growth hormone, interferon (IFN) β and IFNγ. Cell lines and overexpression systems have been extensively used to identify both interacting partners, and the cytokines inhibited by the SOCS proteins. While clearly establishing their potential activities, particularly for therapeutic administration of supraphysiological levels of SOCS proteins [7], this overexpression approach has been relatively unhelpful in identifying physiologically relevant cytokine signalling pathways. For example, while ectopic expression of SOCS3 can inhibit IFNγ signalling [8–10], SOCS3-deficient mice clearly show that although SOCS3 is essential for G-CSF, IL-6, LIF and leptin signalling, it is in fact, dispensable for regulation of IFNγ signalling (Table 1) [11–18].

Mouse models of SOCS1 deficiency are characterised by a complex multi-organ inflammatory infiltrate and demonstrate critical roles for SOCS1 in regulation of toll-like receptor (TLR) signalling, types I and II interferon signalling and γ_c-cytokine-dependent T cell homeostasis [19–26]. Although CIS-deficient mice are anecdotally reported to have no cytokine-related defects [27], transgenic models support a role for selective inhibition of the JAK–Stat5 signalling pathways. CIS-transgenic mice resemble Stat5-deficient mice, with defects in growth and lactation due to reduced growth hormone and prolactin signalling. CIS-transgenic mice additionally display enhanced TCR signalling and impaired responses to IL-2 [28,29]. SOCS2-deficient mice grow to gigantic proportions and display reduced neuronal density and aberrant neuronal differentiation due to perturbations in growth hormone signalling [30–32]. SOCS4-deficient mice have not been reported and SOCS5 and SOCS6-deficient mice do not display an overt phenotype [33,34]. Deletion of the *SOCS7* gene highlights a critical role in regulation of insulin signalling [35].

Functional redundancy may not only explain the obvious lack of effect in CIS, SOCS5 and SOCS6-deficient mice, but also the apparent absence of roles for SOCS proteins in regulation of JAK/STAT dependent cytokines such as erythropoietin (EPO) and thrombopoietin. It is possible that other SOCS family members can compensate for the loss of individual SOCS proteins, a proposition that remains to be formally tested by the generation of mice with compound SOCS deficiencies. The remainder of this review will discuss in detail the mechanics of SOCS action and address some of the complexities inherent in interpretation of the mouse models.

Table 1
SOCS proteins in specific cytokine pathways

SOCS	Critical role in cytokine signalling	References
CIS	Growth hormone, prolactin, IL-2	[28,29]
SOCS1	IFN-γ, IL-2 γ_c, IL-4	[19–26,92,93]
SOCS2	Growth hormone	[30–32]
SOCS3	G-CSF, IL-6, LIF, IL-23, leptin	[11–18,99]
SOCS4	Not known	
SOCS5	IL-4	[69,101,102]
SOCS6	Insulin	[33,34,75]
SOCS7	Insulin	[35]

2. SOCS biochemistry—mechanism of action

Much of our understanding of SOCS function is derived from studies of SOCS1 and SOCS3. These two related SOCS proteins have many similarities as well as some intriguing differences. Both can block signalling by direct inhibition of JAK enzymatic activity yet apparently require different anchoring points within the receptor complex (Fig. 1). While the primary SOCS1 interaction is with a critical phosphotyrosine residue located within the JAK catalytic loop (Y1007 in JAK2) [36], SOCS1 has also been reported to interact with phosphotyrosine residues on the IFNAR1 and IFNGR1 subunits in a JAK1-independent manner [37,38]. The SOCS3–SH2 domain was also initially shown to interact with Y1007 in JAK2, albeit with

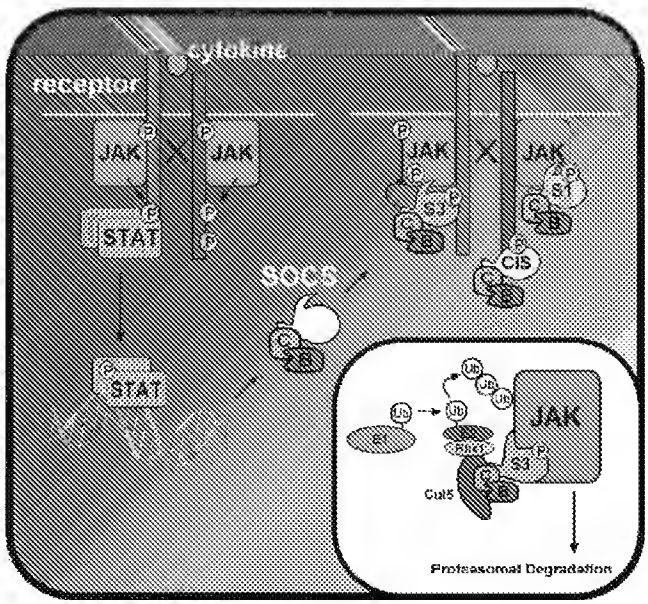

Fig. 1. Mechanism of SOCS action. In general, cytokine binding to a cognate receptor results in activation of the JAK/STAT pathway and induction of *Socs* gene transcription in a STAT-dependent manner. The SOCS proteins then inhibit signalling either by direct inhibition of JAK kinase activity (SOCS1: S1), SH2-recruitment to the receptor cytoplasmic domain, followed by inhibition of JAK activity (SOCS3: S3), or by competition with STAT–SH2 domains for specific receptor phosphotyrosine residues (CIS, SOCS2). An additional level of regulation is provided by an E3 ubiquitin-ligase complex bound to the SOCS box motif, which ubiquitinates the associated proteins targeting them for proteasomal degradation (inset).

Table 2
SOCS–SH2 domain interactions

SOCS	SH2 domain target	Tyrosine residue	References
CIS	EPO R	Y401	[130]
	PRL R	Y532	[131]
	leptin R	Y985, Y1077	[67]
SOCS1	JAK	Y1007 (JAK2)	[2]
	IFNGR1	Y441	[28]
SOCS2	GH R	Y487, Y595	[111]
	Leptin R	Y1077	[67]
SOCS3	Leptin R	Y1138	[133]
	EPO R	Y401	[133]
	gp130	Y757	[134]
	G-CSF R	Y729	[135]
	IL-12 Rβ2	Y800	[136]
SOCS4	EGFR	Y1092	[45]
SOCS5	Not known		
SOCS6/7	IRS2	Y823[a]	[34]
	IRS4	Y700, Y828, Y921, Y959[a]	[34]

[a] Predicted based on binding preferences.

slightly lower affinity [39], but subsequent studies demonstrated a high affinity interaction with phosphotyrosine residues located within receptor subunits (Table 2). The majority of these sites are also binding sites for the tyrosine phosphatase SHP2, leaving the interpretation of early studies based on mutation of receptor tyrosine residues ambiguous [40]. CIS and SOCS2 also bind to receptor phosphotyrosines and inhibit signalling by competing with STAT molecules for recruitment to the receptor complex (Fig. 1) (Table 2).

2.1. Inhibition of signalling—the role of the kinase inhibitory region (KIR)

The KIR is required for inhibition of JAK kinase activity, and is located N-terminal and adjacent to the SH2 domain in SOCS1 and SOCS3. This 12 amino acid region is proposed to act as a pseudo-substrate, lodging in the catalytic cleft to block further JAK enzymatic activity, a hypothesis supported by KIR point mutations that abrogate SOCS action without affecting SH2 domain binding [36,39]. More recently a SOCS1-KIR peptide and Tkip, a SOCS1 analogue, have been shown to interact directly with the JAK autophosphorylation loop and inhibit IFN-γ signalling in primary cells [41,42]. SOCS5 has also been suggested to have a putative KIR [43] and while untested, this region is strikingly similar to Tkip (Fig. 2). While the *in vitro* studies offer an alternative mechanism for KIR/JAK interaction they may not reflect the primary biological interaction. Exactly how the KIR inhibits JAK activity will no doubt be clarified once a crystal structure of the SOCS/JAK complex becomes available.

Given that SOCS1 and SOCS3 can interact with both receptor and JAK, a two-step interaction model can also be envisaged, whereby the SOCS1/3–SH2 domain is first recruited to the receptor cytoplasmic domain and subsequent bi-modal binding to JAK through the SH2 domain and KIR results in a high affinity interaction, inhibition of JAK enzymatic activity and potential proteasomal degradation.

2.2. A unique SOCS SH2 domain

Mutagenesis studies identified small regions at the N-termini of the SOCS1 and SOCS3–SH2 domains, and at the C-terminus of the SOCS3–SH2 domain, which were critical for phosphotyrosine binding. Defined as an N- and C-extended SH2 domain (N-ESS and C-ESS, respectively) [36,39], this was somewhat unusual, given

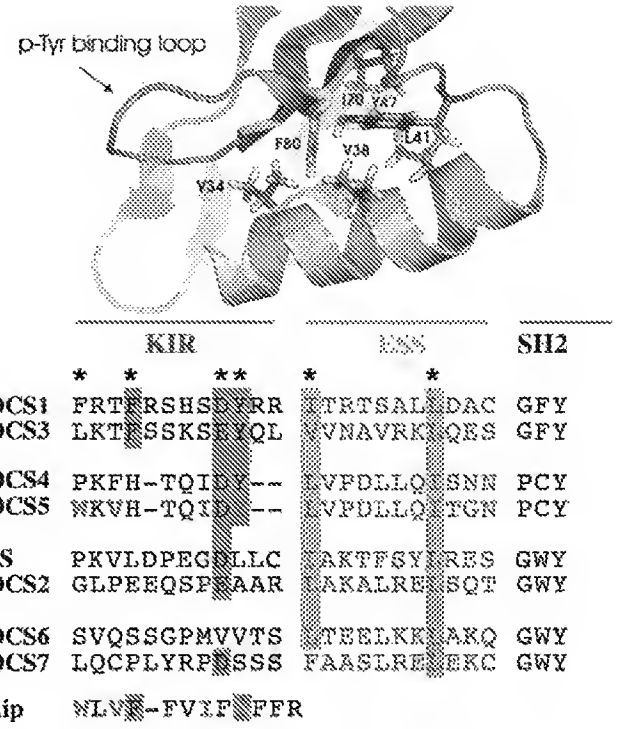

Fig. 2. A unique SH2 domain. Top panel: ribbon diagram of the SOCS3 SH2-domain showing interaction of the ESS (grey) with the phosphotyrosine binding loop (reproduced with permission from Elsevier Press) [44]. Bottom panel: amino acid alignment encompassing KIR, ESS and N-SH2 domain boundaries and highlighting residues critical for SOCS1 and SOCS3 function (*), residues conserved between SOCS1, 3, 4, 5 and Tkip (blue background), conserved between CIS, SOCS1–7 (green background), or conserved between SOCS5 and Tkip (purple font).

the perceived modular nature of the domain. The solution of the SOCS2, 3 and 4–SH2 domain structures [44–46] now offers an explanation for these results. The N-ESS forms a 15-residue alpha helix, which directly contacts the phosphotyrosine-binding loop and determines its orientation. For example, in SOCS3 the conserved Val38 and Leu41 form strong bonds with Phe80 and Ile70 and predictably when mutated, disrupt N-ESS interaction with the phosphotyrosine binding loop [39,44]. Conservation of these key residues suggests that the N-ESS is likely to be a common structural feature of this class of SH2 domains (Fig. 2).

The C-ESS is in fact an intrinsic structural component of the SOCS3–SH2 domain that is spatially displaced by a 35-residue unstructured PEST insertion located between two secondary structural elements, the αβ helix and the BG loop [44]. PEST sequences are rich in proline (P), glutamate (E), serine (S) and threonine (T) and are thought to signal for rapid proteolytic degradation [47,48]. It is therefore not surprising that deletion of the SOCS3 PEST sequence stabilises SOCS3 expression [44]. As several other SOCS proteins contain putative PEST sequences [49] this may prove to be a common mechanism for regulation of SOCS protein levels.

2.3. The SOCS box motif

The greater SOCS family is defined by a 40 amino acid SOCS box motif, which in the majority of cases, is located at the C-terminus of the protein. The SOCS family now encompasses more than 40 proteins and can be further subdivided based on the relevant protein-interaction domain. These include SPRY domains (SSB), ankyrin repeats (ASB), WD-40 domains (WSB), and GTPases [50,51]. The SOCS box consists of three α-helices bound to an E3 ubiquitin ligase complex that together with an E1 ubiquitin-activating

enzyme, and an E2 ubiquitin-conjugating enzyme results in the polyubiquitination and proteasomal degradation of SOCS binding partners. The first helix mediates an interaction with elongin C, while elongin B stabilises the complex making limited contacts with the loop region between helix 2 and 3 [45,46,52]. The active ligase consists of elongins B/C, the ring-finger protein Rbx1, and Cullin-5. An interesting aspect of the SOCS2-B/C structure is the interaction between the C-terminus of the SOCS2–SOCS box and the N-ESS of the SH2 domain [46], suggesting that the SOCS box may also contribute to SH2 domain stability. Notably, the SOCS box tail is extended in SOCS4, 5 and 7, and while the current SOCS4-elongin BC structure suggests an alternative domain arrangement with extensive interaction between the extended C-terminal tail and N-terminal region, this construct still retains an N-terminal sequence tag which participates in the SOCS box interaction [45].

While the SOCS1–SOCS box has been demonstrated in a number of instances to ubiquitinate putative targets such as JAK2, a TEL–JAK2 fusion, VAV and IRS proteins, directing their subsequent degradation through the proteosome [53–55], in very few cases have SOCS proteins been shown to ubiquitinate the receptor complex. An exception is a recent publication showing SOCS3-dependent ubiquitination of the G-CSF-R on Lys632, which in this instance is required for intracellular trafficking of the G-CSF-R from the early endosomes to lysosomes [56]. Regardless, the SOCS1 and SOCS3–SOCS boxes are clearly required *in vivo*, as mice lacking either SOCS box respectively display enhanced IFN-γ and G-CSF signalling, albeit to a lesser degree than mice completely deficient in either protein [57,58].

2.4. Post-translational regulation of SOCS protein expression

In many instances cytokine induction of SOCS protein expression has been shown to be STAT-dependent [6,59,60]. SOCS1 and SOCS3 are rapidly induced following cytokine stimulation and are promptly degraded on cessation of signalling, indicating that protein half-life is precisely regulated. Several mechanisms have been proposed to regulate SOCS expression. The presence of a PEST sequence in SOCS3 appears to mediate non-proteasomal degradation, while SOCS box-dependent ubiquitination of SOCS3 on lysine 6, at least *in vitro*, contributes to proteasomal degradation of the SOCS3 protein [61].

SOCS3 is uniquely phosphorylated within the SOCS box on Tyr204 and Tyr221 and this appears to have dual consequence; interaction with the elongin B/C complex is lost, destabilising the SOCS3 protein, and signalling through the Ras-MAP kinase pathway can be potentiated by interaction with p120 RasGAP [62,63]. While these, and other, *in vitro* studies demonstrate that SOCS3 can be potently phosphorylated by the EGFR, PDGFR, Src and JAK kinases [64–66], the full biological implications of phosphorylation remain to be explored. The modulation of SOCS3 stability by elevated EGFR and Src kinase activity may have the net effect of potentiating oncogenic proliferation in certain cancers.

Another form of cross-modulation is the concept of SOCS–SOCS regulation. Using a mammalian protein-interaction strategy (MAPPIT) Piessevaux and colleagues have shown that SOCS2, 6 and 7 can interact with the SOCS box motif inhibiting the function of the target SOCS protein. They argue that as the SOCS2 and SOCS6–SOCS boxes are also required for SOCS–SOCS inhibition, this indicates proteasomal degradation of the target SOCS protein [67,68].

2.5. The N-terminus of SOCS4–7

In general, SOCS proteins such as CIS and SOCS2, can function by blocking access to phosphotyrosine residues and targeting proteins for ubiquitination and proteasomal degradation. Although it is tempting to extrapolate this well-defined function to other members of the SOCS family, it is evident that a unique mode of receptor recruitment may be involved for SOCS5 [69–71]. Most striking are the extended N-terminal regions of SOCS4 (270 aa), SOCS5 (368 aa), SOCS6 (369 aa) and SOCS7 (385 aa) (excluding the ESS), suggesting these four proteins form a sub-group within the SOCS family.

2.6. SOCS4 and SOCS5

SOCS4 has remained the most elusive of the SOCS proteins, with little information available regarding its expression, regulation or function. SOCS4 and SOCS5 share greater sequence homology with each other than with other members of the SOCS family [50], with conservation largely restricted to the SH2 domain (92% amino acid identity) and suggesting that while the SH2 domains may have an overlapping binding specificity [50], the N-terminal regions will have unique protein targets.

Early *in vitro* studies suggested that SOCS4 and SOCS5 might regulate EGF signalling [70,71]. Two modes of interaction with the EGFR have thus far been identified; a phosphorylation-independent interaction via the SOCS5 N-terminal region and a phosphorylation-dependent interaction via the SOCS4–SH2 domain and Tyr1092 in the EGF-R cytoplasmic domain [45,70,71]. In aggressive hepatocellular carcinoma, there is an inverse relationship between EGF-R expression and SOCS4 and SOCS5 expression [72]. This regulation of EGF-R signalling seems likely to reflect a conserved evolutionary function as the SOCS4/5 *Drosophila* homologue SOCS36E has also been shown to inhibit EGF-R signalling [73].

SOCS5 is also thought to regulate IL-4 signalling and this is discussed within the context of T helper (Th)1/Th2 cell polarisation (Section 3.5).

2.7. SOCS6 and SOCS7

SOCS6 and SOCS7 again share greater sequence identity with each other than with other members of the SOCS family (56% within the SH2 domains) and their expression appears to be co-regulated in response to insulin signalling. A role in insulin signalling is also supported by SOCS6/7 interaction with the insulin receptor, PI3K p85 subunit, and IRS2/4 proteins [34,74]. Although SOCS6-transgenic mice displayed enhanced glucose metabolism [75], this was not supported by gene-targeting experiments. SOCS6-deficient mice appeared largely normal, with only a mild retardation in growth [34]. Deletion of the SOCS7 gene had a more dramatic effect, resulting in premature death due to hydrocephalus on a C57BL/6 background, with no obvious defects in glucose homeostasis. Conversely, on a 129/SvJ background, SOCS7-deficient mice survived and enhanced insulin signalling was associated with improved glucose tolerance [35,76]. This cautionary example highlights the impact strain background can have on the manifestation of knockout phenotypes.

SOCS7 was first identified through its ability to interact with the SH3 domain of the adaptor protein NCK and is unique in its possession of a proline-rich N-terminus and nuclear localisation signal [77]. Most recently, Kremer et al. [78] have identified a link between SOCS7 and regulation of the actin cytoskeleton, showing that SOCS7 interacts with both septin proteins and NCK, shuttling NCK into the nucleus following DNA damage and activating p53 and cell-cycle arrest.

3. The complexities of SOCS biology

3.1. SOCS1 and innate immune responses

Activation of macrophages in response to pathological stimuli often relies on the integration of signals provided by cytokines and

microbial compounds such as LPS. However, when macrophages are chronically exposed to LPS, they become unresponsive to cytokines such as IFNγ, normally a potent activating stimulus. SOCS1 and SOCS3 are induced by diverse mechanisms in macrophages in response to microbial products and may be responsible for suppressing JAK/STAT signalling. Thus SOCS proteins not only provide a mechanism for the innate immune system to prevent an excessive response to pathogenic challenge, but may also inhibit macrophage function during chronic antigen exposure.

Microbial activation of TLRs results in the activation of NFκB, IRF-3, IRF-7 and MAP kinase pathways leading to the induction of thousands of genes, including the interferon signalling pathways. While a role for SOCS1 in regulation of IFN signalling has been definitively established [19], a role for SOCS1 in directly regulating TLR signalling remains controversial. LPS activation of TLR4 induces SOCS1 expression, an effect partially mediated by MyD88 and type I interferon signalling [79]. The sensitivity of SOCS1-deficient mice to sub-lethal doses of LPS is dependent on Stat1, indicating a key role for IFN in mediating this effect [23,24]. SOCS1 is also important for LPS tolerance in splenic adherent cells and resident macrophages, regulating macrophage activation and cytokine secretion upon secondary exposure to LPS [23,24]. In contrast, Gingras et al. [25] used bone marrow-derived macrophages to demonstrate that SOCS1 was not required for mediating LPS tolerance or for regulating LPS-induced nitric oxide production, NFκB or MAP kinase activation, but was instead regulating IFNβ-induced JAK/STAT activation. A role for SOCS1 in regulating type I interferon signalling and responses to viral infection was further confirmed in subsequent studies [37]. In some studies, SOCS1 overexpression inhibited LPS-induced production of nitric oxide and TNFα via interaction with IRAK1 [23,24], but this was not confirmed by others [79]. Kinjyo et al. [23] demonstrated increased JNK, p38 and NFκB activation in response to LPS, as well as increased pStat1 activation in IFNγ/SOCS1-deficient mice, suggesting that SOCS1 may be regulating both primary (TLR) and secondary (IFN) innate immune signalling pathways.

SOCS1 also mediates the polyubiquitination and degradation of TIRAP, a signalling adaptor downstream of TLRs, to prevent excessive p65/RelA phosphorylation and production of IL-6 and TNFα, without affecting IκBα phosphorylation or MAP kinase activation [25,26]. Therefore, in addition to negative regulation of interferon signalling, SOCS1 also has a key role in modulating TIRAP—downstream of TLR1/2, TLR2/6 and TLR4 but not TLR9 (where signalling is independent of TIRAP).

Recent studies have also uncovered a novel function for SOCS2 in innate immunity. SOCS2-induced proteasomal degradation of TRAF6 has been found to be an important mechanism in mediating the anti-inflammatory actions of aspirin-induced lipoxins [80,81].

3.2. SOCS3 regulates LIF receptor signalling

SOCS3-deficient embryos die between 12 and 16 days gestation [27,82] and this was initially reported to result from excessive erythropoiesis due to enhanced EPO signalling [27]. Independent analyses by Roberts et al. [82] confirmed that SOCS3-deficiency was embryonic lethal, but the authors did not detect defects in erythropoiesis or EPO signalling. Rather, lethality was attributed to the poor development of embryonic vessels and maternal sinuses in the labyrinthine layer of the placenta. A tetraploid aggregation assay, resulting in a fully functioning placenta with a wild-type trophoblast layer and a SOCS3-deficient foetal component, generated SOCS3-null embryos that could survive until birth. The mice, however, were smaller than littermates, exhibited cardiac hypertrophy and died within 25 days of birth [16]. Importantly, the embryonic lethality of SOCS3-deficient embryos could also be rescued if mice were deficient in either LIF or the LIF receptor, indicating that SOCS3 is required for modulating LIF signalling in giant trophoblast cells [15,16].

3.3. SOCS3 is a key regulator of inflammation

Mice with a conditional deletion of the *Socs3* gene in hematopoietic and endothelial cells die as young adults due to severe inflammatory lesions in the peritoneal and pleural cavities [11,15]. Administration of G-CSF *in vivo* mimics emergency granulopoiesis during infection. In the absence of SOCS3, this process is grossly exacerbated, with neutrophil infiltration and destruction of liver, lung, muscle and spinal tissue, resulting from increased intensity and duration of G-CSF-induced Stat3 activation [11,18]. An independent study using SOCS3-deficient neutrophils observed a dramatic increase in Bcl-xL, a Stat3-inducible and anti-apoptotic protein, providing a potential mechanism contributing to the enhanced survival of SOCS3-deficient neutrophils [18].

Mice with SOCS3-deficient haemopoiesis are also highly susceptible to inflammatory joint disease [83]. In rheumatoid arthritis (RA) patients, Stat3 and SOCS3 levels are elevated and adenoviral gene transfer of SOCS3 or dominant negative Stat3 reduced both the proliferation of murine RA synovial fibroblasts and the severity of disease in a mouse model [84]. IL-6 has dual roles in the progression of arthritis, decreasing cartilage destruction in the acute phase and increasing joint inflammation in the chronic phase of disease [85,86]. IL-6 and Stat3 phosphorylation are pivotal to the pathology associated with ulcerative colitis and Crohn's disease, and elevated SOCS3 expression again suggests that SOCS3 may also have a regulatory role in these diseases [87]. During wound repair, SOCS3 negatively regulates gp130-dependent signalling in keratinocytes and immune cells, and prevents excessive neutrophil accumulation at wound sites and production of macrophage-secreted MIP-1α [88].

3.4. Interpreting Stat3 activation in the absence of SOCS3

Models of Stat3-deficiency are commonly cited as evidence for a specific function, but often ignore compensatory mechanisms and should be interpreted cautiously [59]. Stat3-deficient embryonic fibroblasts stimulated with IL-6 produce an IFNγ-like anti-viral response due to increased Stat1 activation and up-regulation of IFNγ-inducible genes [89]. Paradoxically, SOCS3-deficient mice, which have enhanced Stat3 activity, display a similar gene transcription profile, with the implication that IFNγ-like responses could contribute to the lethal inflammatory phenotype [12,13]. The resolution of these conflicting observations and exactly how Stat3 prevents activation of an IFNγ-like Stat1-mediated response remains unclear, but has profound implications for the potential therapeutic use of Stat3 inhibitors.

Inhibitors such as SOCS3, that regulate the magnitude and duration of cytokine-induced Stat1 and Stat3 activation, may also affect the balance between IFNγ-like Stat1 responses and IL-6/IL-10-like Stat3 responses [12,13,17]. The functional outcome of prolonged activation is controversial and two contradictory models have been proposed. The absence of SOCS3 has been purported to induce an anti-inflammatory Stat3-dependent, IL-10-like response and alternatively, to induce a pro-inflammatory Stat1-dependent IFNγ-like response. The obvious discord derives from differing methodologies used in the studies. Yasukawa et al. analysed inflammatory cytokine production in macrophage cultures whereas Croker et al. and Roland et al. analysed gene expression profiles in hepatocytes and macrophages [12,13,90]. In cells lacking the gp130- or leptin-R-SOCS3 binding sites, less inflammatory cytokines are produced with a similar profile to that observed for IL-10 stimulation. These phenomena appear to be cell type-specific, occurring in

macrophages but not synovial fibroblasts, and illustrating the likely complexity of the situation *in vivo* [91].

None of the above studies fully explain the lethal inflammation in mice with a conditional deletion of *Socs3* in hematopoietic and endothelial cells. As is evident from examination of gene transcription profiles in SOCS3-deficient cell types, multiple pathways are inappropriately regulated upon cytokine stimulation and the cellular outcome is therefore likely to be complex.

3.5. SOCS proteins in allergy and asthma

Increasing evidence supports a role for SOCS1, SOCS3 and SOCS5 in coordinating T helper (Th)1/Th2 cellular profiles. The lethality of the SOCS1 knockout can be rescued not only by removal of IFN-γ but also by deletion of *Stat6*, indicating that perturbations in IL-4 and/or IL-13 signalling contribute to the fatal inflammatory infiltrates found in SOCS1-deficient mice [19,92]. Consistent with these observations, T cell-specific deletion of *Socs1* results in enhanced production of both IFN-γ and IL-4 and spontaneous differentiation into Th1 and Th2 cells [93]. Recent studies found a correlation between elevated SOCS1 expression and asthma severity in patients, and suggest that SOCS1 may inhibit IFN-γ-dependent Th1 differentiation, thereby enhancing Th2-mediated pathology [94,95]. Given the importance of the Th2 cytokines in asthma and the key role of SOCS1 in regulating IL-4 and IFNγ signalling, it is not yet clear how therapeutic modulation of SOCS1 function would affect disease progression.

SOCS3 is preferentially expressed in Th2 cells and elevated expression levels are observed in patients with asthma and atopic dermatitis [69,96,97]. Although expression of a *Socs3* transgene promoted Th2 responses, conditional deletion of the *Socs3* gene did not, as predicted, enhance Th1 polarisation, but instead revealed a subtle role for SOCS3 as a negative regulator of the Th3-like (Treg) subset with increased IL-10 and TGFβ production [97,98]. Conditional deletion of *SOCS3* has also revealed a critical role as a negative regulator of IL-23 signalling, resulting in enhanced Th17 polarisation [99].

SOCS5 is differentially expressed in Th2 cells, and although no abnormalities in Th1/Th2 differentiation are noted in SOCS5-deficient mice [33,69,100], increasing evidence supports a role for SOCS5 in Th cell differentiation. Seki and colleagues suggest SOCS5 interacts via its N-terminus with the IL-4 receptor alpha (IL-4Rα) Box 1 region, blocking JAK1 association and subsequent Th2 differentiation. Indeed, expression of a T cell-specific SOCS5 transgene augments the Th1 response in mouse models of bacterial sepsis and allergic conjunctivitis [69,101,102]. Conversely, global expression of a SOCS5 transgene results in enhanced Th2 responses following OVA sensitisation and challenge [103]. Given the caveats associated with transgenic expression of SOCS5 it seems likely that the lack of a SOCS5 knockout phenotype reflects either functional redundancy within the IL-4 signalling cascade or a more complex role for SOCS5 in T helper cell biology.

3.6. SOCS2 and growth hormone signalling

Growth hormone (GH) is a key regulator of postnatal somatic growth, and signals primarily through the JAK2–STAT5b pathway. GH induces expression of several SOCS family members (CIS, SOCS1-3), suggesting that SOCS proteins may regulate GH signalling [104,105]. While each of these SOCS proteins have been shown to interact with the GH receptor (GHR) and when overexpressed interfere with the JAK2–STAT5b pathway (Table 2) [106–109], it is only SOCS2 that is thought to play an important physiological role in regulation of GH action. Unlike other SOCS knockouts, the phenotype of SOCS2-deficient mice resembles that of GH-transgenic mice, displaying increased body weight consequent upon enhanced bone size and an enlargement of most organs [30,110]. These mice also exhibit a hypersensitive growth response triggered by exposure to exogenous GH [111]. Further, GH-induced STAT5b activation is prolonged in SOCS2-deficient hepatocytes, consistent with the finding that the SOCS2-deficient phenotype is dependent on STAT5b [112]. This evidence indicates that SOCS2 is an important negative regulator of GH actions [113,114].

Paradoxically, high concentrations of SOCS2 have been found to positively regulate growth hormone signalling in cell lines and transgenic mice [104,108,109]. While the effects of high SOCS2 expression could be explained by SOCS2 inhibition of other SOCS proteins [68], a major caveat to interpretation of these results is whether the cellular concentration in these artificial systems can be achieved physiologically.

3.7. SOCS proteins and cancer—the consequences of de-regulated SOCS expression

Dysregulation of the JAK/STAT signalling pathway has been implicated in malignant progression. Many human cancers including hepatocellular carcinoma (HCC), non-small-cell lung cancer, mesothelioma, head and neck squamous cell carcinoma (HNSCC), cholangiocarcinoma, Barrett's adenocarcinoma, and myeloproliferative diseases, demonstrate constitutive STAT phosphorylation, and this is frequently accompanied by hypermethylation of one or more *Socs* genes [115–121]. In the case of HNSCC, high rates of *Socs3* methylation correlate with higher grades of dysplasia [121]. In addition, *Socs1* methylation has been associated with transformation of liver cirrhosis to HCC [114,122]. These observations strongly suggest that SOCS proteins may be tumour suppressors. Consistent with this notion, experimental overexpression of SOCS proteins in cancer cells reduces STAT activity, inhibits proliferation and induces apoptosis of these cells [114,117,120,121]. Loss of SOCS expression may therefore facilitate or favour tumour progression in alliance with other oncogenes. However, the mechanism that induces *Socs* methylation is unclear.

In contrast, persistent expression of SOCS1 and/or SOCS3 is observed in several haematological malignancies such as cutaneous T-cell lymphoma (CTCL), chronic myeloid leukemia (CML), ALK+ anaplastic large cell lymphoma (ALCL), and some acute leukemias. In these circumstances, heightened expression coincides with constitutive activation of JAK/STAT pathways [123–127]. One possible explanation is that within the cancer microenvironment, haemopoietic tumour cells are sustained by an array of cytokines, which constantly activate JAK/STAT pathways to support cancer cell growth and survival. Expression of SOCS proteins may be a natural consequence of this. In these tumours, failure of other negative regulatory pathways acting upon the JAK/STAT pathway or inappropriate regulation of oncogene expression or perturbed oncogene function such as the TEL–JAK2 fusion protein, may well be present, overwhelming the capacity of SOCS proteins to dampen STAT activation. Under these conditions, the inhibitory action of SOCS proteins may not have a significant impact on cancer cell proliferation and survival.

Interestingly, a number of studies have demonstrated that SOCS1 and/or SOCS3 expression in chronic myeloid leukemia (CML) or CTCL is inversely correlated with sensitivity to IFNα [125,127–129], a therapeutic cytokine with anti-tumour activity. When overexpressed in CTCL cells, SOCS3 suppressed IFNα-induced Stat1 and Stat3 phosphorylation and reduced the growth inhibitory effect. In addition, suppression of SOCS3 expression (by STAT3 knockdown), increased IFNα sensitivity by 40% [128]. Whether SOCS3 directly modulates the sensitivity of tumour cells to IFNα in a physiological context remains unknown. Collectively,

these data suggest that perturbed SOCS expression may contribute to the malignant phenotype and favour disease progression, rather than being an early event in the oncogenic process.

4. Concluding remarks

During the past decade the SOCS proteins have been revealed as key negative regulators of cytokine and growth factor signalling. The generation of mice lacking individual *Socs* genes has been instrumental in defining the role of individual SOCS proteins in specific cytokine pathways and without doubt, future studies will address the issue of functional redundancy. While gene-targeting studies have highlighted critical roles for the SOCS proteins, the complexity of the mouse models, particularly with regard to immune function and inflammation, suggests that these studies should be carefully interpreted, and certainly more work is required before we can predict the consequences of using SOCS proteins or SOCS agonists/antagonists in a clinical setting. Although a great deal of work remains to clarify the role of SOCS proteins in human disease, we are hopeful that therapeutic modulation of SOCS expression and function will be beneficial in the treatment of many human diseases such as arthritis, sepsis, allergy and cancer.

The intention of this review was to provide an introductory overview and stimulate the audience to explore the field further. While we have endeavoured to include relevant publications we apologise to any authors who have been omitted due to space constraints. By necessity, we have not discussed some key aspects of SOCS biology, such as roles in obesity and mammary gland development.

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